

1986 EMS Abstracts 63

167

USE OF BrdU LABELING IN THE HUMAN LYMPHOCYTE HGPRT SOMATIC MUTATION ASSAY
P.Ostrosky-Wegman, R. Montero, M.Colin* and C. Cortinas de Nava. Instituto
de Investigaciones Biomédicas. Apdo. Postal 70228 Ciudad Universitaria
04510 México, D. F.

Using the autoradiographic assay proposed by Strauss and Albertini to quantitate human *in vivo* somatic mutation at the hypoxanthine guanine phosphoribosyl transferase locus (HGPRT) we enumerated lymphocytes able to incorporate tritiated thymidine in the presence of 6-thioguanine and determined the variant frequencies of this gene in cryopreserved lymphocytes from 15 normal human blood samples. The median V_F value was 4.8×10^{-6} with a range of $0-22.2 \times 10^{-6}$. 13 of the values were less than 6.0×10^{-6} , while 2 were greater than 15×10^{-6} . According to Albertini et al., values of 15×10^{-6} or greater should be scored as elevated V_F 's, nevertheless larger samples should be studied to set normal values.

Practical considerations, including economic factors, led us to look for an alternative method to detect HGPRT mutants. To recognize interphase cells that have replicated DNA, we used BrdU instead of tritiated thymidine as proposed by Latt and Tice. The results obtained suggest that techniques used for sister chromatid differentiation could be applied with some modifications to detect putative HGPRT mutants and to determine variant frequencies in the HGPRT locus making this assay available to any cytogenetic laboratory.

(Mutat. Res. 61: 353, 1979; Abstracts E.M.S. 14th annual meeting 120, 1983
J. Cell Biol. 62: 546, 1974; Exp. Cell Res. 102: 232, 1970)

168

CONVERSION OF AZIDE TO A MUTAGENIC METABOLITE BY O-ACETYL SERINE SULFHYDRYLASE ISOLATED FROM CICER ARIETINUM. W. M. Owais, S. R. Hazza*, and A. Hunaiti*, Yarmouk University, Irbid, Jordan

O-acetylserine sulfhydrylase isolated from seven-day-old seedlings of chick peas (*Cicer arietinum*) was purified to apparent homogeneity by ammonium sulfate precipitation, gel filtration, affinity chromatography and polyacrylamide gel electrophoresis. The purified enzyme was able to convert sodium azide to a mutagenic metabolite in the presence of O-acetylserine. This metabolite increases the frequency of histidine reversion in *Salmonella typhimurium* TA 1530 strain. The isolated enzyme has identical k_m and k_i values for azide (N_3^-) which strongly suggests that azide and the natural substrate (S^-) use the same catalytic site on the enzyme. Results from this study provide further supportive evidence for the role of O-acetylserine sulfhydrylase in the conversion of azide to the mutagenic metabolite.

169

PROTECTION OF E. COLI AND S. TYPHIMURIUM BY MICROMOLAR LEVELS OF EXOGENOUS REDUCED GLUTATHIONE (GSH). Roland A. Owens¹, Dick C. Kuo and Rebecca M. Bjornson. Department of Biology, The Johns Hopkins University, Baltimore, MD 21218. ¹Present address: Laboratory of Developmental Pharmacology, Rm. 6C-101, Bldg. 10, National Institutes of Child Health and Human Development, Bethesda, MD 20892.

The addition of 26 μ M reduced glutathione (GSH) to cultures of *S. typhimurium* strain TA1534 partially protects the bacteria from the toxic effects of 54 μ M N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) as evidenced by alleviation of MNNG-induced growth delay. Preincubation of MNNG with equimolar GSH neutralizes MNNG mutagenicity. Addition of micromolar GSH to cultures of a GSH *E. coli* mutant strain protects the bacteria from growth inhibition induced by micromolar levels of mercuric chloride, methyl mercuric chloride, silver nitrate, cisplatin, cadmium chloride, cadmium sulfate, and iodoacetamide, respectively. Mercuric chloride, cisplatin, MNNG, silver nitrate and iodoacetamide form stable reaction products with GSH detectable by paper chromatography. Equivalent micromolar concentrations of oxidized glutathione (GSSG) provide little or no protection and form no reaction products detectable by paper chromatography. Export of about 50% of synthesized GSH, leading to μ M levels of external GSH in the media of some bacterial stock cultures (Owens & Hartman 1985 Environ. Mut. 7:Suppl. 3:47), may be an important defense mechanism against an array of environmental agents that otherwise would be toxic to enteric bacteria when the environmental agents are present in the media at micromolar levels. (Supported in part by grant ES03217 to P.E. Hartman).